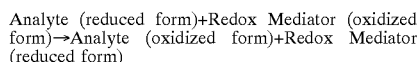


[0055] The geometric relationship between the electrodes may also vary. For example, the electrodes may be aligned in any order capable of generating the desired detection current. Likewise, each electrode may be positioned at any desired angle to the flow of the test sample. In the embodiments shown in FIGS. 3-5, the substrates 40 and 80 are laminated such that the working electrode 42 is positioned adjacent to the reference electrode 46, the reference electrode 46 is positioned adjacent to the calibration working electrode 44, and the calibration working electrode 44 is positioned adjacent to the counter electrode 48. Moreover, each electrode is also positioned perpendicular to the flow of the test sample. Of course, various other electrode configuration and/or geometric relationships may be utilized in the present invention.

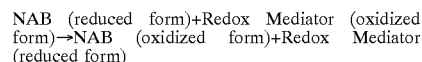
[0056] Regardless of the particular configuration of the electrodes, the present inventors have discovered that the use of separate substrates 40 and 80 may substantially reduce background interference. Specifically, the use of separate substrates allows the working electrodes 42 and 44 to be treated with affinity reagents without regard to potential contamination of the reference and counter electrodes 46 and 48 that would otherwise cause background interference. Once the treatment process is complete, the reference and counter electrodes 46 and 48 may then be placed in the desired location. Although not required, the laminated substrates 40 and 80 may be laminated in the desired position using any well-sealing technique, such as by adhesively attaching the substrates 40 and 80 with glue, double-sided tape, and so forth. The extent that the substrates 40 and 80 are laminated together may vary as desired. For example, in some embodiments, the entire periphery of the substrates 40 and 80 are adhesively attached.

[0057] Various embodiments of detection the presence of an analyte within a test sample using the assay device 20 of FIG. 4 or 5 will now be described in more detail. It should be understood, however, that the embodiments discussed below are only exemplary, and that other detection techniques and assay device configurations are also contemplated by the present invention. To initiate the detection of an analyte within a test sample, a user may simply apply the test sample to the sample pad 21 through which it may then travel. From the sample pad 21, the test sample then travels to the conjugate pad 22 where any analyte within the test sample mixes with and attaches to a redox label. In one embodiment, for instance, the label is horseradish peroxidase (HRP) and the analyte of interest is glucose. Because the conjugate pad 22 is in fluid communication with the sample channel 14, the labeled analyte may migrate from the conjugate pad 22 to the channel 14 through which it travels for the desired amount of time until it reaches the detection working electrode 42, where the labeled analyte binds to the specific binding capture ligand and reacts with a redox mediator. In one embodiment, for example, the analyte is reacted as follows:



[0058] In addition, non-specific binding may be monitored and corrected using the optional calibration working electrode 44. It is intended that the amount of non-analyte materials that bind to the calibration working electrode 44 will be similar to the amount of non-analyte material that

non-specifically binds to the detection working electrode 42. Thus, in this manner, the background signal due to non-specific binding may be compensated. In one embodiment, for example, the non-analyte biological materials (abbreviated "NAB") are reacted as follows:



[0059] Detection techniques, such as amperometric, coulometric, voltammetric, etc., may then be used to detect the analyte. A further description of such electrochemical detection techniques is described in *Electrochemical Methods*, A. J. Bard and L. R. Faulner, John Wiley & Sons (1980). In one embodiment, for example, a potentiostat or reader may apply a potential difference between the detection working electrode 42 and counter electrode 46. When the potential difference is applied, the amount of the oxidized form of the redox mediator at the counter electrode 46 and the potential difference is sufficient to cause diffusion limited electro-oxidation of the reduced form of the redox mediator at the surface of the detection working electrode 42. The magnitude of the required potential is thus dependent on the redox mediator. Namely, the potential is typically large enough to drive the electrochemical reaction to or near completion, but not large enough to induce significant electrochemical reaction of interferents, such as urate, ascorbate, and acetaminophen, that may affect the current measurements. Similarly, the potential difference may also be supplied between the optional calibration working electrode 44 and counter electrode 46. When the potential difference is applied, diffusion limited electro-oxidation of the reduced form of the redox mediator occurs at the surface of the calibration working electrode 44.

[0060] Generally, the detection and calibration working electrodes 42 and 44 simultaneously generate a respective signal from a single measurement of a sample. The simultaneously generated signals are averaged by a processing circuit, such as a multi-channel potentiostat. Multi-channel potentiostats are well known in the art, and are described, for instance, in U.S. Pat. No. 5,672,256 to Yee, which is incorporated herein in its entirety by reference thereto for all purposes. Each channel of a multi-channel potentiostat may function as a potentiostat, and thus may be associated with its own reference and/or counter electrode, or may share reference and/or counter electrodes. One suitable example of a multi-channel potentiostat that may be used in the present invention is commercially available under the name "MSTAT" from Arbin Instruments, Inc. of College Station, Tex. Once detected, the current measured at the detection working electrode 42 is calibrated by the current measured at the calibration working electrode 44 to obtain a calibrated current reading that may be correlated to the concentration of analyte in the sample. The correlation may result from predetermined empirical data or an algorithm, as is well known in the art. If desired, the generated current and analyte concentration may be plotted as a curve to aid in the correlation therebetween. As a result, calibration and sample testing may be conducted under approximately the same conditions at the same time, thus providing reliable quantitative or semi-quantitative results, with increased sensitivity. In the case of a sandwich assay format, the signal provided by the detection working electrode 42 is directly proportional to the analyte concentration in the test sample. In the case of a competitive assay format, which may, for instance,